DATA SHEET

LAROVA
The Nucleotide Company

Hot Start Polymerase

Heat-activatable DNA polymerase for high specificity
Thermus aquaticus, recombinant, *E. coli*

Cat. No.	Amount
HS_100KU	100 kunits
HS_1000KU	1.000 kunits

Unit Definition: One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 70 °C using hering sperm DNA as substrate.

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid

Concentration: 5 units/µl

Description:
Hot Start Polymerase provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The polymerase is recommended for routine PCR applications (up to 4 kb fragment length), high throughput PCR or genotyping.
The Crystal Buffer system guarantees robust and reliable amplification results in almost all PCR applications. The buffer contains a well-balanced ratio of potassium-, ammonium- and magnesium-ions to ensure high specificity and minimal by-product formation without the need of additional optimization steps.
Ruby Buffer additionally contains gel loading buffer and an inherent red dye allowing the direct loading of the PCR product into the gel. The red dye allows an easy visual control during PCR set-up and in combination with the density reagent the direct loading of the reaction product into the gel.
The enzyme replicates DNA at 72 °C. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also possesses a 5'→3' polymerization-dependent exonuclease replacement activity but lacks a 3'→5' exonuclease (proof-reading) activity.

Activation step
Hot Start Polymerase requires no prolonged heating or denaturing step. The polymerase inhibiting aptamer is quickly released at the increased temperature of thermal cycling.

Content:
Hot Stort Polymerase (red cap)
5 units/µl aptamer-inhibited hot start polymerase in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween-20, 0.5 % Nonidet P-40, 50 % (v/v) Glycerol, pH 8.0 (25°C)

Ruby Buffer (black cap)
10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl, $(NH_4)_2SO_4$ and 20 mM $MgCl_2$, red tracking dye and density reagent for gel loading

Crystal Buffer (green cap)
10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl, $(NH_4)_2SO_4$ and 20 mM $MgCl_2$

Assay Set-Up:
Before starting, vortex all components thoroughly to ensure homogeneity.
Prepare a premix for the number of assays you need according to the following protocol:

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comp.	cap	stock conc.	final conc.	1 assay @20 µl	1 assay @ 50 µl
PCR-grade Water	white			fill up to 10 µl	fill up to 30 µl
Ruby Buffer or Crystal Buffer	black or green	10x	1x	2 µl	5 µl
dNTP Mix / 10 mM #NU-1006	white	10 mM	200 µM	0.4 µl	1 µl
Hot Start Polymerase	red	5 units/µl	0.025 units/µl	0.1 µl	0.25 µl
primer mix or each primer		10 µM each primer	200 - 400 nM each primer	0.4-0.8 µl	1 - 2 µl
template /sample DNA				10 µl < 10 ng DNA	20 µl < 20 ng DNA

Select PCR tubes, stipes or plates as recommended for your cycler model.
Aliquot premix into each well and add template DNA (or PCR-grade Water for negative controls).

Cycling Conditions:
Spin down the tubes/plate briefly to remove bubbles and place them into the cycler.

initial denaturation	95 °C	2 min	1x
denaturation annealing[1)] elongation[2)]	95 °C 50 - 68 °C 72 °C	10 - 20 sec 10 - 20 sec 20 sec - 4 min	25 - 35x

[1)]The annealing temperature depends on the melting temperature of the primers used.
[2)]The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

Gel Loading and Down-Stream Applications:
Ruby Buffer includes a density reagent + tracking dye and allows the direct loading of the PCR products into a electrophoresis gel. For DNA detection / fluorescent DNA staining we recommend to use new generations dyes (i.g. SYBR DNA Stain) instead of the classical but highly mutagenic ethidium bromide.
Crystal Buffer is recommended for down-stream applications such as DNA sequencing, ligation, restriction digestion or where an analysis of the PCR product by absorbance or fluorescence excitation is required. For gel electrophoresis add gel loading buffer and fluorescent DNA stain before loading the PCR into the gel. Using pre-stained gels or post-run staining protocols is also possible.

Optimization of $MgCl_2$ concentration:
A final Mg^{2+} concentration of 2.0 mM is recommended in combination with Labeling Buffer. However, if an individual Mg^{2+} optimization is essential add 25 mM $MgCl_2$ stock solution as shown in the table below.

final $MgCl_2$ conc.	**20 µl final assay volume**	**50 µl final assay volume**
2 mM	-	-
3 mM	0.8 µl	2.0 µl
4 mM	1.6 µl	4.0 µl
5 mM	2.4 µl	6.0 µl